

Vegetative Growth and Root Development of *Euonymus Japonica* Cuttings is Influenced by Various Concentrations of Indole Butyric Acid (IBA) and Transplantation Dates

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Abstract

Propagation through cuttings is the expedient way to get more number of plants, moreover root initiation in cuttings of *Euonymus japonica* is a key success if plant growth regulator is applied. Plant growth regulator like IBA stimulates cell division, cell elongation and metabolic activities at the place where cut is made and thus causes root initiation. Hence the current research study was carried out to investigate the influence of different IBA concentrations (0, 2000, 4000, 6000 and 8000ppm) and planting dates (with 15 days of interval) on vegetative and rooting development. Semi hardwood cuttings treated with IBA @ 6000 ppm attained minimum days to sprouting and root initiation, whereas, maximum sprout length, number of leaves sprout⁻¹, stem diameter, total number of leaves plant⁻¹, leaf area, number of roots plant⁻¹, root length and root diameter. Best results on sprout length, number of leaves sprout⁻¹, stem diameter, number of leaves plant⁻¹, leaf area, number of roots plant⁻¹, root length and root diameter and minimum days to sprouting and root initiation were recorded in cuttings planted on 6th March. The interaction effects reported best results at IBA level of 6000 ppm and planting date, 6th March for rooting and growth attributes. It could be concluded that cutting dipped in 6000ppm IBA solution improved most of the studied attributes of *Euonymus japonica*. Similarly, planting *Euonymus japonica* cuttings on 6th March was found best to produce better rooting.

Keywords: Indole-3-butyric acid, Cuttings Time, Adventitious Root Development, Rooting potential, *Euonymus japonica*

1. Introduction

Evergreen or Japanese spindle (*Euonymus japonica* Thunb) is a perennial shrub, native to China, Japan and Korea that belongs to family Celastraceae. More than 130 species within the *Euonymus* genus have been reported with several types of cultivars having many types and shape of leaves from fully green to variegated white leaves (such as *Euonymus japonica*

albomarginata) or variegated yellow leaves (such as *Euonymus japonica aureomarginata*) (Gilman, 1999). Japanese spindle is mostly propagated through cutting usually used for most landscape shrubs plants (Whitcomb, 1978). Implementing propagation methods to enhance transplant success, establishment, and post-plant maintenance is a major objective for plant nurseries involved in the production of shrubs to be used for gardens and natural landscapes in regions with a Mediterranean climate. In this regard, the production of oleander rooted cuttings with a well-developed root system is fundamental for successful transplanting and establishment in the field. By growing plants in a greenhouse or other protected facilities, plant nurseries force plants to bloom out of season. Ornamental shrubs with colors create a great amount of impulse buying from the average garden plant consumer because a flowering plant provides immediate gratification and guarantees that the consumer is getting what they paid for (Pilon, 2005). Rooting potential of stem cutting can be affected by various factors such as time of cutting can be the key factor in rooting response of many plants due to variation in climatic (Blazich, 1987).

Seasonal variation was observed in rooting ability of *Euonymus* species where cuts taken are in two diverse season's spring and summer are easily rooted. Root's initiation was observed in the cuttings that were taken from fully dormant plants in winter season which was rapid and profuse, especially under mist (Tukey and Lee, 1971). Cuttings from the healthy parent's plants ensure vigorous and healthy offspring plants. Softwood stem cuts can be taken from newly growing tips following a flush on the parent plant typically in the summer season; however, this can occur throughout the growing season. Semi-hardwood cuttings are from partially matured wood also available throughout the season and dependent upon species (Blazich, 1987).

For successful propagation adventitious root formation is required. For years, improvement in rooting has been observed in species with the application of rooting hormones. Generally, the attributes of root quality e.g., quantity of roots as well as length has been observed to be improved by the treatment of hormones (Dirr, 1986). Indole-3-butyric acid (IBA) has a wide range of uses in the trades of nursery as a root promoting chemical accompanied by 1-naphthaleneacetic acid (NAA), due to less toxicity even at different levels (Ruppert, 1974). Therefore, mentioned root promoting hormone helps in the stimulation of adventitious root development after cuttings (Hartmann et al., 2002). The hormones can be applied in various forms including the solid or liquid in solution as after dissolving in solvent or water. (Ruppert, 1974; Whitcomb, 1978). The concentration of IBA depends upon the species as, more concentration could be needed if propagation takes place by softwood cutting because little amount could not have prominent effect and vice versa. Basically, most of the shrubs and evergreen have shown good rooting performance with the application at the rate of 2000 to 4000 ppm (Ruppert, 1974). Root promoting compounds such as rooting hormones are used to increase the percentage of cuttings which form roots, reduce the time to root initiation, increase number of roots produced per cutting and to increase uniformity of rooting. Indole-3-butyric acid and 1-naphthalenacetic acid are commonly used in commercial propagation because of their consistency in promoting adventitious root formation on cuttings (Boyer and Graves, 2009). Keeping in view the importance of propagation through cuttings, this project was designed to study the effect of various concentration of IBA and timing of plantation alone or in combination on rooting and growth performance of *Euonymus japonica*

2. Materials and Methods

2.1. Experimental site and procedure

The research experiment was carried out at New Developmental Farm, Horticulture Section, The University of Agriculture, Peshawar Pakistan, classified as semiarid and the maximum wind speed is 35 km/h. It located at 34.01° N latitude, 71.35° E longitude at an altitude of 350m above sea level in Peshawar valley with a sub-tropical climate (Ahmad et al. 2019). Peshawar is located approximately 1600 km north of the Indian Ocean. The research farm is irrigated by the Warsak canal from river Kabul (Alam et al. 2020). Both the summer and winter weathers are extreme (Basit et al. 2019a), characterized by severe winter and hot prolonged summer where the average minimum temperature during winter is 5 °C while during summer, the average maximum temperature reaches up to 45 °C. The wettest month (with the highest rainfall) is March (78 mm) and driest month (with the lowest rainfall) is June (7 mm) approximately (Sajid et al. 2020). The experiment was laid out in Complete Randomized Design having two factors repeated three times. Semi hardwood cutting of *Euonymus japonica* having three-node cuttings (length 10–12 cm) were dipped for 30 seconds in different concentration of Indole butyric acid (IBA) i.e. 0, 2000, 4000, 6000 and 8000ppm and then planted on different planting dates (6th March, 21 March and 5th April) under plastic tunnel in polyethylene tubes having the mixture of sand, silt and clay at 1:1:1.

2.2. Preparation Method of IBA solution

In order to prepare different IBA concentrations for treatment of cuttings, first of all the stock solution of subsequent concentration of IBA were prepared. The stock solution was prepared by dissolving 4g of IBA in ethanol (100 ml) and then the solution having volume of 500 ml was prepared by adding distilled water.

2.3. Data Collection of various attributes

Data was collected for different rooting and vegetative attributes *Euonymus japonica*. Days to sprouting (cutting date till sprouting were counted), Sprout length (cm), Number of leaves sprout⁻¹, Number of leaves plant⁻¹, Leaf area (leaf area of the leaves in every treatment was measured with the help of leaf area meter in cm²), Stem diameter (The stem thickness was measured by using of vernier caliper for every treatment in mm), Days to root initiation (Root initiation was recording from cutting date till roots emergence), Number of root plant⁻¹, Root length (cm) and Root diameter (data for root diameter was calculated in each treatment with the help of vernier caliper in mm).

2.4. Statistical Analysis

Mean data taken for different parameters of different replications was subjected to Analysis of Variance (ANOVA) suitable for Randomized Complete Block Design (Gilani *et al.*, 2018) using statistical software package (Statistix 8.1, Inc, Tallahassee FL, USA) Basit *et al.* (2018). The productivity and quality criteria was carried out by using bivariate analysis of variance and LSD test was used for means comparison at $P \leq 0.05$ (Jan *et al.*, 2009)

3. Results and Discussion

3.1.Days to sprouting.

Days to sprouting was affected significantly by different concentration of IBA and planting dates alone and in combination (Table 1). Dipping of cutting in different concentration of IBA from 0 to 6000ppm significantly reduced days to sprouting from 34.3 to 18.7 beyond this range, days to sprouting was increased to 19.2 when cuttings were dipped in 8000ppm solution of IBA (Figure 1A). Similarly, minimum days to sprouting of cuttings in respect to cutting dates were observed in the cuttings planted on 6th March (25.5), while maximum days to sprouting was noted in the cuttings planted on 21st March (27.4) (Figure 2A). Regarding interaction, late sprouting was observed in untreated (control plants) cuttings planted on 5th April as compared to the days to sprouting (17.7) cutting dipped in 6000ppm solution of IBA and planted on 6th March (Figure 3A). The IBA concentration had significantly reduced days to sprouting of the cuttings of *Euonymus japonica* which might be attributed due to role of IBA in root induction and thus cause early sprouting of the cuttings. Our results are in conformity with the findings of Souidan *et al.* (1995) who observed positive effect of IBA concentrations days to sprouting. The results of the present study are in conformity with Fragoso *et al.* (2017) who stated that IBA concentrations exhibited a gradual increase in the rooting Japanese flowering cherry. The same results were also reported earlier by Siddiqui and Hussain (2007) reported that IBA increased rooting in *Ficus Hawaii* and thus helped the sprouting of cuttings. The earlier sprouting in the cuttings might be exhibited due to the already present differentiated cells that sprout directly without any further differentiation while in control where no IBA treatment was used there had an inhibitory or delaying effect on rooting. This might be because of the dry matter or the presence of phyto-toxicity which inhibited or reduced the growth. This was confirmed by Kareem *et al.* (2016).

Table 1. Mean Square value of growth and rooting related traits of *Euonymus japonica* as influenced by IBA concentration and time of plantation of cuttings.

SOV	DF	Mean Sqaure (MS)								
		DTS	SL	NOLPS	NOLPP	LA	SD	DTRI	NORPP	RL
Cutting Dates (D)	2	12.42*	0.42***	2.06*	271.8***	7.03*	0.06*	20.06***	581.422***	8.52*
IBA Concentration (C)	4	468.07**	2.44***	20.3*	748.75***	4.28 ^{ns}	0.46***	206.66***	1874.48***	66.28*
C × D	8	7.81*	0.02***	1.06 ^{ns}	65.68 ^{ns}	0.13 ^{ns}	0.01 ^{ns}	3.15*	63.8111*	0.4*
Error	30	2.64	0	0.44	32.42	1.91	0.01	1	26.1778	0.8
Total	44									

DTS:Days to sprouting; SL:Sprout length; NOLPS:Number of leaves per sprout; NOLPP:Number of leaves per plant; LA: Leaf area; SD:Stem diameter; DTRI:Days to

root induction; NORPP: Number of roots per plant; RL: Root length; RD: Root diameter

*****: $p \leq 0.01$; *: $p \leq 0.05$; ns: Non significant**

3.2. Sprout length (cm)

According to results presented in Table 1 indicated that there is a significant difference between IBA concentrations and cutting dates alone and in their interaction (Table 1). The mean data showed that maximum sprout length was observed in the cuttings treated with 6000 ppm of IBA concentrations (2.6cm) followed by cuttings treated with 8000 ppm of IBA concentrations (2.5cm), whereas the minimum sprout length (1.5cm) was observed in control treatment (Figure 1A). Compared with the means of planting dates, the cuttings planted on 6th March had produced 60% higher sprout length as compared to the cuttings planted on 5th April (Figure 2A). Similarly, the highest value of sprout length (2.7cm) was recorded in cutting dipped in 6000ppm IBA solution that were planted on 6th March and cutting in control treatment planted on 5th April had attained lowest value of sprout length (1.7cm) (Figure 3B). Cuttings treated with 6000 ppm of IBA concentrations significantly increased the sprout length of *Euonymus japonica* might be due to role of IBA which increased the number of roots and resulted more nutrients uptake as a result got higher sprout length (Siddiqui and Hussain, 2007). The same phenomenon was also explained by some other researchers that either IBA concentration or different cutting dates could resulted better rooting as compared to control (Reddy and Reddy, 1990; Navjot and Kahlon, 2002; Hore and Sen, 1993; Scaloppi and Martins, 2004; Ram *et al.*, 2005). IBA concentration can be explained by the fact that the same concentration supported better root quality (length and numbers), this increased surface area for nutrient absorption from below ground parts to the above ground parts (Leakey *et al.*, 1990; Gilani *et al.*, 2019). This trend of results on shoot length can be explained by the same mechanism that influences shoot numbers that is enhancement of mineral nutrients transport by applied IBA to the growing points of the cutting (Akwaatulira *et al.*, 2011). Since the same concentration has supported more number of shoots, basically an increase in the number of shoots means more surface area for photosynthesis. More assimilates can translate to an increase in metabolic processes responsible for shoot proliferation (Gilani *et al.*, 2019).

3.3. Number of leaves sprout⁻¹

It is obvious from Table 1 that there is highly significant difference among IBA concentrations, cutting dates and their interaction. The maximum number of leaves per sprout (7.3) was observed in the cuttings treated with 6000 ppm of IBA concentrations followed by 6.4 and 5.7 number of leaves sprout⁻¹ recorded in the cuttings treated with 8000 ppm and 4000 ppm of IBA concentrations, respectively. Whereas the minimum number of leaves per sprout (3.4) was observed in control treatment. Similarly, maximum number of leaves per sprout (5.9) was observed in the cuttings planted on 6th March, while cuttings planted on 21st March had attained minimum number of leaves sprout⁻¹ (5.2) (Figure 2B). Regarding interaction, the highest value of number of leaves sprout⁻¹ (8.0) was observed in cutting treated with 6000 ppm IBA compared to those remained untreated and planted on 5th April (2.3) (Figure 3C).

IBA concentrations and cutting dates significantly increased the number of leaves per sprout. This might be attributed due to application of IBA which induced more roots and increased the amount of nutrient uptake as result produced a greater number of leaves per sprout (Basit *et al.*, 2019b; Ram *et al.*, 2005). Moreover, IBA increase the movement of materials into the vascular vessels (Tayz and Zayger 2006) and the transport of carbohydrates from leaves to root (Fathi and Esmailpor 1999) and also the nutrients uptake from roots to leaves (Siddiqui and Hussain 2007). Similar results are also in conformity with Ucler *et al.*, (2004) reporting that IBA concentrations at 6000 ppm and also the cutting dates affects the rooting of the cuttings significantly and could resulted better plant growth and development from cuttings.

3.4. Number of leaves plant⁻¹

Variation in number of leaves plant⁻¹ were observed in IBA concentrations and cutting dates except their interaction which had non-significant influence on number of leaves plant⁻¹ (Table 1). An increase in number of leaves plant⁻¹ from 20.8 to 43.6 were observed when dipping concentration of IBA was increased from 0 to 6000 and then shows a reduction in number of leaves up to (39.0) in cuttings dipped in 8000ppm IBA solution (Figure 1B). Similarly, reduction in number of leaves (14.59 and 29.37%) were observed when planting was delayed from 6th March to 5th April (Figure 2B). The number of leaves per cutting of *Euonymus japonica* was significantly affected by cutting dates and IBA concentrations. Maximum number of leaves in IBA treatment might be due to the increased number of leaves and roots by IBA application. Our results are also in correspondence with the findings of other researchers Chinnu *et al.*, 2012 and Beaura *et al.*, 2007). The cuttings treated with 6000 ppm of IBA concentration increase the number of leaves (Ucler *et al.*, 2004) and improve the buds growth and the development of branches (Blythe *et al.*, 2004, Khoshkhoy *et al.*, 2002).

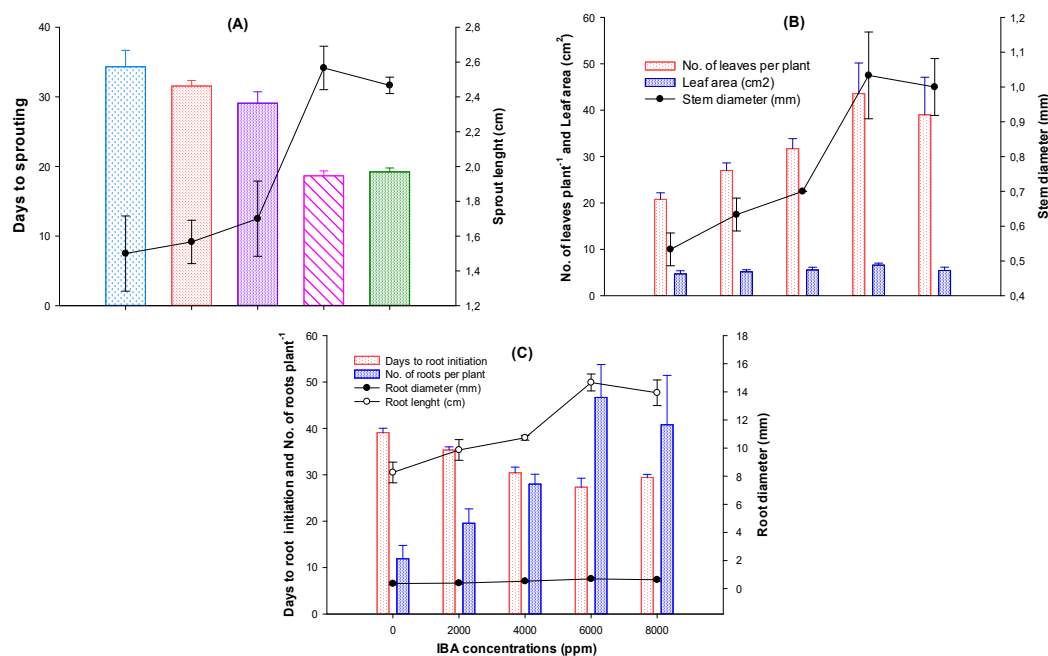


Figure 1. Growth and rooting related attributes of *Euonymus japonica* as influenced by IBA concentrations.

3.5. Leaf area (cm²)

Leaf area was significantly influenced by cutting dates except IBA concentration and their interaction of IBA concentrations and cutting dates which had non-significant influence on leaf area (Table 1). However, the maximum leaf area (6.6 cm²) was observed in the cuttings dipped in 6000 ppm of IBA solution followed by 5.6 and 5.5 cm² in the cuttings obtained in cutting treated with 4000 ppm and 8000 ppm of IBA concentrations, while the minimum leaf area (4.7 cm²) was observed in control treatment (Figure 1B). Decrease in leaf area from 6.2 to 4.8 cm² were observed when plantation was delayed from 6th March to 5th April (Figure 2). Leaf area was significantly affected by the IBA concentrations. IBA at 6000 ppm of concentration increased the leaf area of *Euonymus japonica*. The increase of leaf area is directly related with the plant growth. The vigorous plant growth gave more leaf area whereas the weak plant growth could result small leaves with lesser leaf area. The cuttings treated with 6000 ppm of IBA concentrations had better plant roots and shoot development (Siddiqui and Hussain 2007). The IBA treated cuttings showed better number of leaves and better stem diameter. It allows the plant to grow and develop more efficiently as compared with the control (Fragoso *et al.*, 2017). The availability of the nutrient due to more roots and better flow of the nutrients in the vascular bundles (Ram *et al.*, 2005) due to the treatment of IBA which helped the plant to grow (Tayz and Zayger 2006) and develop more leaf area than control.

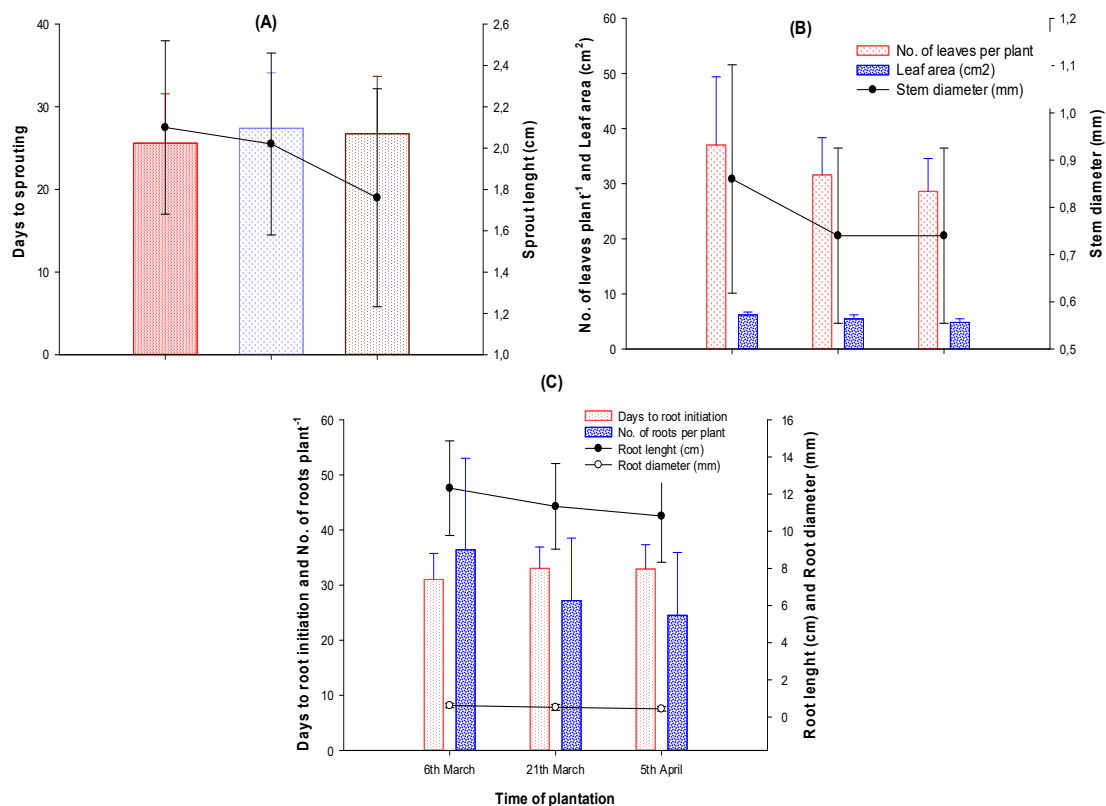


Figure 2. Growth and rooting related attributes of *Euonymus japonica* as influenced by time of planting of cuttings.

3.6. Stem diameter (cm)

Various IBA concentrations and different cutting dates resulted highly significant difference for stem diameter (Table 1). Dipping of cuttings in different IBA solution had significantly higher stem diameter compared to control treatment (Figure 1B). Similarly decrease in stem diameter (22.22 and 11.11%) were recorded when plantation of the cuttings was delayed up to 5th April (Figure 2B).

IBA at 6000 ppm of concentrations has increased the stem diameter which might be due to more development of roots as result increase the nutrients uptake (Ucler *et al.*, 2004) and the development of leaves is processed. Leaves undergoes photosynthesis and the carbohydrates could be easily flow in the vascular bundles along with other nutrients from the roots (Fathi and Esmailpor 1999) as result increased the stem diameter. IBA provide a smooth pathway for the distribution of the nutrients in the plant tissues (Tayz and Zayger 2006). The results of the present study are also in conformity with Reddy and Reddy (1990) and also Navjot and Kahlon, (2002) who reported that IBA and cutting dates could contributed better role in the development and the induction of roots as compared to control treatment.

3.7. Days to root initiation

IBA concentration, cutting dates and their interaction had resulted significant difference on days to sprouting (Table 1). Decrease in days to root initiation (39.1 to 27.3) was observed when IBA concentration was increase from 0 to 6000ppm (Figure 1C). Among cutting dates, 6.77 and 6.12% increase in days to root initiation was observed when plantation of cutting was delayed from 6th March to 5th April (Figure 2C). Similarly, late root initiation (40.3) was observed in cuttings of control treatment when on 5th April as compared to cutting dipped in 6000ppm IBA solution and planted on 6th March (24.7) (Figure 3D). The reduction in the initiation of roots depends upon the treatment application and cutting times (Polat and Caliskan 2009). IBA (Auxins) improve the root initiation, root quality and number of branches in cuttings (Hoseini 2014). Similar results were also recorded by Last *et al.* (1991) and Rose *et al.* (1992) that IBA could induced roots in the cuttings better as compared to control. Rahman *et al.* (2002) also confirmed that the cuttings treated with IBA concentration induced early rooting in the cuttings and gave vigor growth to the plant. Briccoli (1989) and Muller (2005) reported that besides the IBA concentrations, the cutting dates and time also affects the induction of roots in the cuttings. The increase of IBA concentration was accompanied by the decreased rooting percentage, suggesting that high IBA concentrations were not suitable for the root formation process (Singh *et al.*, 2003). Although IBA increases the elasticity of cell wall, accelerating division, excessive concentration of hormones may inhibit this process (Rahman *et al.*, 2000). Exogenous auxin application in layers during period of active growth (high endogenous auxin levels) could raise the hormone levels above optimal concentrations, leading to decrease in rooting (Moreira *et al.*, 2009).

3.8. Number of roots plant⁻¹

IBA concentrations, cutting dates and their interaction significantly affected number of number of roots per plant (Table 1C). Increasing trend of number of roots per plant (11.9 to 46.7) was recorded with increasing the concentration of IBA from 0 to 6000ppm after that a decrease in number of roots per plant was observed in the cuttings treated with 8000 ppm of

IBA. Regarding cutting dates, (32.69%) decrease in number of roots per plant was recorded in cutting planted on 5th April as compared to those planted on 6th March (Figure 2C, 3E). It is evident from the results that the cuttings treated with 6000 ppm of IBA concentrations significantly increased the number of roots per plant but beyond this level root number decreased. The results of the present study were confirmed by Khajehpour *et al.*, (2014) who reported that IBA concentration increase the number of rooting but the increase of IBA concentrations could partially affects both the roots and the growth of shoot and could not play an active role in rooting of cutting. It reduced the number of roots and also the uptake of the nutrients which do not allow the plant to grow properly. The optimum dose of the IBA could improve the number of roots. The results of the present study were also confirmed by Souidan *et al.* (1995).

3.9. Root length (cm)

Root length was significantly influenced by various IBA concentrations and different cutting dates except their interaction (Table 1). Significant increase in root length was recorded with increasing IBA concentration up to 6000ppm after that decrease in root length was observed. The maximum root length (14.7cm) was noted in the cuttings treated with 6000 ppm of IBA concentrations which was statistically at par with root length (13.9cm) in the cuttings treated with 8000 ppm of IBA solution, whereas the minimum root length (8.3) was observed in control treatment (Figure 1C). Among cutting dates, the longest root length (12.3cm) was observed in the cuttings planted on 6th March as compared to cuttings planted on 5th April (Figure 2C). The root length of *Euonymus japonica* increased in the early sown and the cuttings treated with 6000 ppm of IBA concentration. This increase in the root length might be due to the influence of IBA (growth regulators) which translocate the metabolites and undergoes metabolism of carbohydrates that might be involved in the hormonal role which increase root length (Siddiqui and Hussain 2007). The same results were also reported by Kareem *et al.* (2016). They explained the role of IBA in increasing cell wall plasticity and cell division which stimulates callus development and root growth (Weaver, 1972). Similarly, Hammo *et al.* (2015) observed that the 3000 mg per liter IBA causes significantly increase in rooting percentage (68.42%), roots number (5.91 root/plant), longest root (3.36 cm), roots dry weight (0.31g) in stem cutting of *Ligustrum ovalifolium*. Auxin induces root formation by breaking root apical dominance induced by cytokinin (Cline, 2000). Seyedi *et al.* (2014) noted that stem cuttings of *Bougainvillea glabra* L. treated with IBA hormone concentrations at 4000ppm induced maximum rooting (93%), root length (36.0cm) and root number (9.0). The results trend on root length can be explained by the fact that proteins from IBA break hydrogen bonds between cellulose micro fibrils promoting cell wall loosening and cells will eventually elongate (Kumar et al., 2015; Qu Yang et al., 2015; Cosgrove, 2000). At optimal exogenous IBA, the rate of cambium de-differentiation is increased, accelerated hydrolytic activity and enhanced callus formation which ultimately gives better root length (Li et al., 2009; Gilani et al., 2019).

3.10. Root diameter (mm)

It is obvious from Table 1 that IBA concentrations and cutting dates significantly affected the root diameter, whereas their interaction was found non-significant. The results revealed that

cuttings treated with 6000 ppm of IBA concentrations exhibited maximum root diameter (0.7mm) as compared to control treatment (0.4mm) (Figure 1C). Furthermore, cuttings planted on 6th March exhibited highest root diameter (0.6) compared to cutting planted on 5th April (0.5) (Figure 2C).

Root diameter was significantly affected by IBA concentration which might be due to reason that root diameter increased in the early cuttings treated with 6000 ppm of IBA concentration. This might be due the gaining of optimum root length achieved by the cutting treated with 6000 ppm of IBA concentration in which the carbohydrates metabolites were more vigorously that play an important role in the increasing of root length. The increase in root diameter might be due to more vegetative growth and accumulation of carbohydrates. Similarly, in semi-hard wood cuttings of olive, percent rooted cuttings, number of primary roots and secondary roots and diameter were maximum in cuttings treated with IBA at 5000 ppm (Thakur *et al.*, 2016).

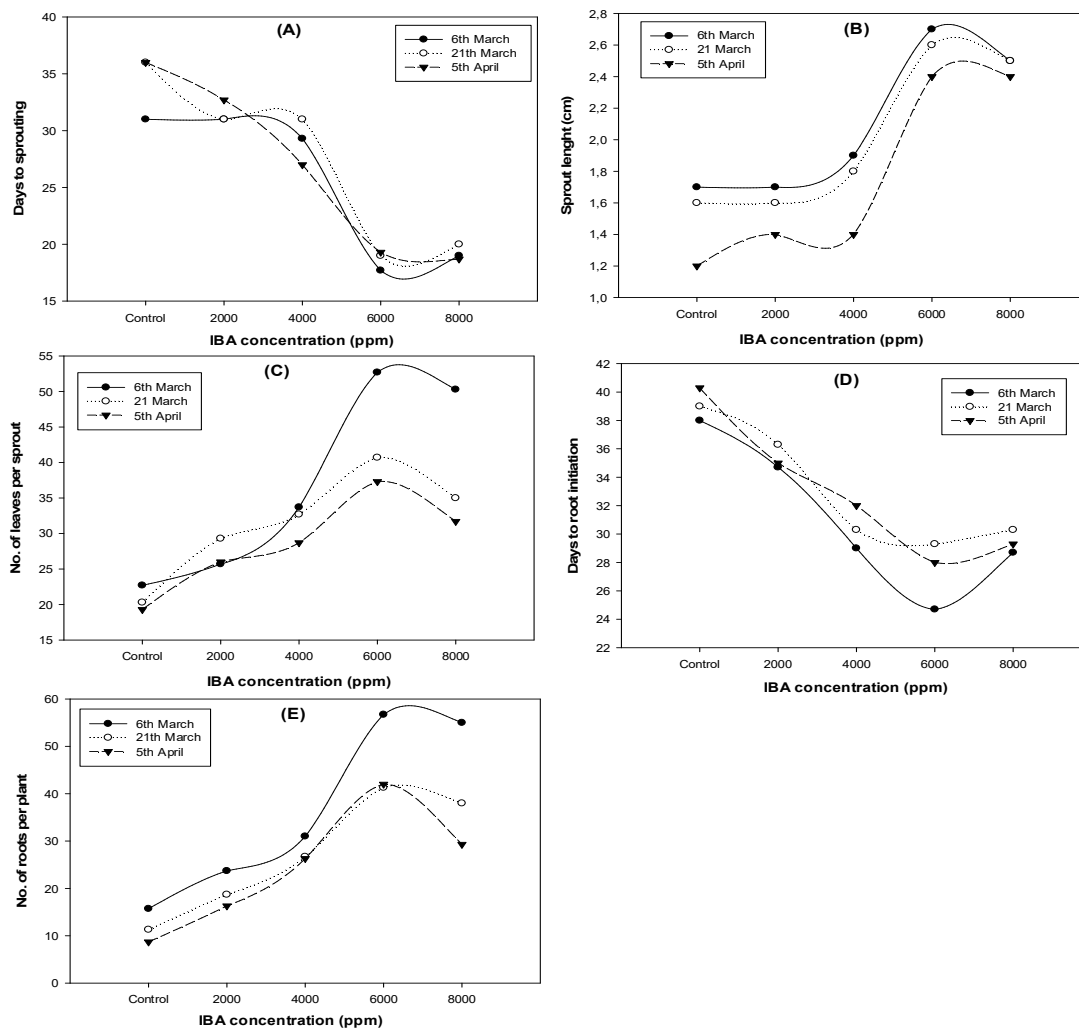


Figure 3. Growth and rooting related attributes of *Euonymus japonica* as influenced by interactive effect of IBA concentration and time of planting of cuttings

4. Conclusion

Use of plant growth regulator especially auxin is essential for proper rooting since it promotes root cutting growth and quality of all traits as compared to untreated cuttings. It was concluded from results that the application cuttings treated with 6000 ppm of IBA solution had resulted maximum sprouting and root development as compared to other concentration of IBA. The cuttings planted on 6th March had the better plant development and root initiation compared to other planting times. Treatment of *Euonymus japonica* cuttings dipped in 6000ppm IBA solution and planted early on 6th March could be best time of plantation for better rooting of cuttings. Therefore, it is highly recommended to use IBA for transplants of cutting under low tunnel condition of *Euonymus japonica* plant thus ensuring the proper rooting of cuttings for earlier transplanting.

5. Author's Contribution

Rafiullah, F. Wahid: Conceived and designed the study.

Rafiullah: Performed the experiment.

S.T. Shah, I. Ullah: Analyzed the data.

A. Basit, I. Ali, S.T. Shah, M. Sajid, I. Ahmad, I.A. Khan: Contributed the chemical/ materials/ analysis tools.

A. Basit: Wrote and reviewed the original manuscript.

References

1. Ahmad M, Khattak MR, Jadoon SA, Rab A, Basit A, Ullah I, Khalid MA, Ullah I, Shair M (2019) Influence of zinc sulphate on flowering and seed production of flax (*Linum usitatissimum* L.): a medicinal flowering plant. Int J Biosci 14:464-476
2. Akbar, B. 2017. Influence of zinc sulphate conc. and its time of foliar application on growth and production of Tuberose. M.Sc (Hons) Thesis. The University of Agriculture Peshawar.
3. Akwatulira F, Gwali S, Okullo, JBL, Ssegawa P, Tumwebaze SB, Mbwambo JR, Muchugi A (2011). Influence of rooting media and indole-3-butyric acid (IBA) concentration on rooting and shoot formation of *Warburgia ugandensis* stem cuttings. African Journal of Plant Science. 5(8):421-429.
4. Alam M, Hayat K, Ullah I, Sajid M, Ahmad M, Basit A, Ahmad I, Muhammad A, Akbar S, Hussain Z (2020) Improving okra (*Abelmoschus esculentus* L.) growth and yield by mitigating drought through exogenous application of salicylic acid. Fres Environ Bulle 29:529-535.
5. Basit, A., K. Shah, M. U. Rahman, L. Xing, X. Zuo, M. Han, N. Alam, F. Khan, I. Ahmed and M. A. Khalid. 2018. Salicylic acid an emerging growth and flower inducing hormone in marigold (*Tagetes* sp. L.). Pure and Applied Biology. 7(4): 1301-1308. <http://dx.doi.org/10.19045/bspab.2018.700151>
6. Basit A, Khan S, Sulaiman Shah S, Shah AA. 2019a Morphological features of various selected tree species on the greater university campus Peshawar, Pakistan. Int J Bot Studies 4:92-97.
7. Basit, A., I. Ullah, S.T. Shah, I. Ullah, N. Alam and S.A.Q. Gilani. 2019b. Effect of Media Amendments on In vivo Root and Shoot Organogenesis of Stevia (*Stevia Rebaudiana*). Int. J. Biosci. 14(4): 55-63.
8. Boyer, N.Z., Graves, W.R. (2009): NAA is more effective than IBA for rooting stem cuttings of two *Nyssa* spp. Journal of Environmental Horticulture 27(3): 183-187.

9. Cline, M.G. (2000). Execution of the auxin replacement apical dominance experiment in temperate woody species. *American Journal Botany*, 87:182-190.
10. Cosgrove DJ (2000). Loosening of plant cell walls by expansins. *Nature* 407(6802):321.
11. Kumar V, Singh MK, Kumar M, Prakash S, Kumar A, Rao S, Malik S (2015). Effect of different doses of Iba and Rooting Media on Rooting of Stem Cutting of Lemon (*Citrus Limon* Burm) cv. Pant Lemon-1. *Journal of Plant Development Sciences*. 7(7): 587-591.
12. Gilani SAQ, Shah K, Ahmed I, Basit A, Sajid M, Bano AS, Shahid U (2019). Influence of indole butyric acid (IBA) concentrations on air layerage in guava (*Psidium guajava* L.) cv. Sufeda. *Pure and Applied Biology* 8(1): 355-362.
13. Hammo, Y.H., Kareem, B.Z.A., Salih, M.I. (2015). Effect of planting media and IBA concentration on rotting ability of stem cutting of *Ligustrum ovalifolium*. *Zanco. Sukhjit Kaur / J. Appl. & Nat. Sci.* 9(1): 173-180.
14. Leakey RR, Mesen JFT, Tchoundjeu Z, Longman KA, Dick JM, Newton A, Muthoka PN (1990). Low-technology techniques for the vegetative propagation of tropical trees. *The Commonwealth Forestry Review* 69(3): 247-257.
15. Li X, Suzuki T, Sasakawa H (2009). Promotion of root elongation and ion uptake in rice seedlings by 4, 4, 4-trifluoro-3-(indole-3-) butyric acid. *Soil Science and Plant Nutrition* 55(3):385-393.
16. Moreira, O., Martins, J., Silva, L. and Moura, M. (2009). Propagation of the endangered Azorean cherry *Prunus azorica* using stem cuttings and air layering. *ARHIPÉLAGO Ciências Biológicas e Marinhas*, 26:9-14.
17. OuYang F, Wang J, Li Y (2015). Effects of cutting size and exogenous hormone treatment on rooting of shoot cuttings in Norway spruce [*Picea abies* (L.) Karst.]. *New forests* 46(1):91-105
18. Pilon, P. *Perennial Solutions: A Grower's Guide to Perennial Production*, 1st ed.; Ball Publishing: Batavia, IL, USA, 2005; p. 546.
19. Rahman, N., Hussain, I., Imran, M., Jan, T. and Awan A. (2000). Effect of different concentrations of IBA on rooting of litchi (*Litchi chinensis*) in air layering. *Pakistan Journal of Biological Sciences*. 3: 330-331.
20. Sajid M, Basit A, Ullah Z, Shah ST, Ullah I, Mohamed HI, Ullah I (2020) Chitosan-based foliar application modulated the yield and biochemical attributes of peach (*Prunus persica* L.) cv. Early Grand. *Bull Nat Res Centre* 44:150. <https://doi.org/10.1186/s42269-020-00405-w>
21. Seyedi, A., Esmacili, A., Ali Zadeh, K.N. and Porsiabidi, M.M. (2014). Comparative evaluation of the rooting in cuttings in (*Bougainvillea glabra* L.) *International Journal of Farming and Allied Sciences*. 3(8): 872-875.
22. Siddiqui MI and Hussain SA (2002). Effect of indole butyric acid and types of cutting on root initiation of *Ficus hawaii*. *Sarhad J Agric* 23(4): 919-926.
23. Singh, S., Kumar, P. and Ansari, S.A. (2003). A simple method for the large-scale propagation of *Dendrocalamus asper*. *Sci. Hortic-Amsterdam*, 100: 251-255.
24. Thakur, M., Sharma, D.D., Babita and Verma, P. (2016). Effect of pre-conditioning treatments and auxins on the rooting of semi-hardwood cuttings of Olive planted during winter under mist condition. *Current World Environment*, 11(2): 560-566.